REMARKS

The suggestions made by Examiner Robinson have been adopted in amending the claims. Claims 1-7 and 10-14 have been amended as shown; formerly proposed claims 15-23 have been modified so as to read on producing a protein as required. Applicants assume that the Office appreciates that the invention does not lie in any particular way of producing the required fusion protein used to identify the soluble domain whose production is claimed. Therefore, the step in claim 1, which requires preparing two or more DNA fragments, has been deleted. The process can begin with simply expressing nucleotide sequences that encode fusion proteins which are composed of fragments of a starting protein fused to a functional protein.

Thus, in all cases, the claims have been amended to obviate the rejections made under 35 U.S.C. § 112, paragraphs 1 and 2. The invention does not reside in the particulars of ligating DNA fragments to obtain nucleic acids for the expression of fusion proteins, *but rather in the recognition that the functionality of a functional portion of the fusion protein is retained only if the remainder of the fusion represents a soluble domain (of an original starting protein)*. In order to conform to the restriction requirement, all claims now require the extra step that the soluble domain actually be produced.

Thus, the claims as presently proposed focus on the central concept of the invention – that the behavior of the functional portion of a fusion protein is directly coupled to a particular characteristic of the amino acid sequence to which it is fused. The fused amino acid sequence will permit the functional protein portion to retain its function if and only if the remaining amino acid sequence represents a soluble protein when divorced from the fusion. Whether this amino acid sequence is a fragment of an insoluble or soluble protein, it in any case represents a soluble domain.

Thus, by producing this fusion protein, either in host cells, such as *E. coli,* or in a cell-free system, by identifying those fusions that retain the functionality of the functional protein, it can be concluded that the attached amino acid sequence represents a soluble domain – a fragment of the protein which is now soluble even if the starting protein is not. This, then, represents a domain that is worthy of study to analyze *in vivo* functions and search for inhibitors as set forth on page 11 of the specification, lines 12-14. *Again, it is emphasized that the claims now all require actual synthesis of the soluble domain thus conforming the claims to the original elected invention.*

The claims as proposed are supported by the claims as originally presented; they are simply limited, as to active steps, to assessment of the function of the functional protein and synthesis of the identified soluble domain.

The amendments to claim 1 delete the irrelevant step of preparing fragments of the DNA encoding an original protein and assume that the expression vector has already been prepared for production of the fusion protein. Other amendments have been made for clarification and to complete essential steps. The amendments to claims 5, 7 and 10 are also for clarification.

Claim 11, as originally presented, appeared to lack a complete description of the claimed subject matter and this has been altered by amendment. New claim 15 is an alternative expression of the subject matter of former claim 1. Support for new claims 21-23 is found in former claim 10; digestion from one of the two possible ends is supported in Figure 1 and Example 2.

No new matter has been added, the claims conform to the elected invention, and entry of the amendment is respectfully requested.

Formal Matters

Applicants appreciate the acknowledgement of the receipt of formal drawings. The requisite papers and disc in compliance with 37 C.F.R. § 1.821 have been submitted in this case on 23 May 2002. In a telephone conversation with the Examiner, our Legal Assistant has confirmed that this sequence listing has been received and has been entered.

The Rejection Under 35 U.S.C. § 112, First Paragraph

All claims were rejected, apparently based on an asserted lack of adequate written description. Applicants are not entirely clear as to what specific defects need to be addressed. The Office first states that the DNA set forth in the claims is described solely by function and not structure. It is not clear whether this is a basis for rejection. In the event that it is, applicants point out that the method of the invention may be employed using the DNA encoding any starting protein; there is a plethora of sequences associated with the many proteins available in the art. Similarly, DNA encoding various functional proteins is also well known. There should be no need to spell out any specific nucleotide sequence, since the method is broadly applicable to any starting protein and any functional protein.

The Office goes on to state that in claim 1 the "essential" method step of ligating the DNA is omitted. The claims presently proposed do not require this as an "essential" step; the ligation will already have been performed when the method steps now claimed are conducted.

With regard to the asserted lack of an "essential step" in transiting from step (c), to step (d) referred to at the bottom of page 3 of the Office action, applicants recognize that there will indeed be activities required by the practitioner in order to ultimately synthesize the soluble domain that was identified to be included in the fusion protein in step (b); however, these steps are not part of the invention. There is a multiplicity of ways these steps could be performed. For example, the fusion protein could itself be isolated and the amino acid sequence determined; it

could be provided with a cleavage site so that the fragment obtained from the starting material could be separately analyzed. Alternatively, the nucleotide sequence encoding the successful fusion protein could be recovered and the structure of the soluble domain determined from the nucleotide sequence. In either case, one might construct an expression vector for the soluble domain itself using the sequence information, or alternatively, the expression vector could be obtained by manipulating the expression vector for the fusion protein. It does not matter to the invention what pathway is chosen to provide a means to synthesize the soluble domain once identified, nor does it matter how the soluble domain is synthesized – e.g. by recombinant means or by solid phase or solution phase synthesis from amino acid components. These steps are not "essential" to the invention. They are activities within the ordinary skill of the art which the skilled practitioner would understand need to be performed.

Claim 10 has been amended in response to the comments made by the Office. First, it has been clarified that it does not matter whether the method involves fragments of an insoluble protein or fragments of a soluble one. The identified fragments must still be soluble. Step (a) has been clarified to indicate the vector indeed encodes a fusion protein. As to the asserted omission of essential steps between (b) and (e), applicants are unsure what these essential method steps might be. The assertion that step (a) does not guarantee that one would be left with a soluble protein and be able to synthesize it in a cell-free system is not understood. It is believed that the amendments to this claim provide sufficient clarification.

As noted, an initial vector is prepared and then partially digested to obtain two or more DNA fragments of the vector. These fragments of the vector contain deletions of the nucleotide sequence encoding the first (starting) protein from which the soluble domain is to be obtained. Now, these fragments are transformed into *E. coli* and those clones of *E. coli* which contain

fragments of the vectors where the deleted form of the first protein is soluble will fluoresce. These are isolated in step (d) and the DNA recovered in step (e). As noted previously, the intervening activities between steps (e) and (f) can be performed in a variety of ways well understood in the art and not relevant to the invention It is believed that claim 10 as amended is responsive to this criticism.

With respect to the criticism of "GFP or derivative thereof" this language has been altered to conform to the specification at page 9, lines 6-13. As noted, there are many known "variants" of green fluorescent protein ("GFP") of various colors.

All claims were also rejected under 35 U.S.C. § 112, first paragraph, as assertedly lacking enablement. The Office outlines several criteria in judging enablement in which it is asserted the claims fall short. The first is said to be the quantity of experimentation necessary, but the discussion under this heading does not appear to relate to the quantity of experimentation, but rather criticism of claim wording as lacking essential steps. It is not clear what essential steps are omitted, and this is not spelled out. Nevertheless, this basis for rejection has been obviated by amendment to the claims and the discussion set forth above with respect to activities that do not form part of the invention.

The second criterion has to do with the breadth of the claims, the amount of direction or guidance presented and the presence or absence of working examples.

Here, the criticism is apparently solely directed to "an unspecified amount of GFP derivatives." Again, the Office is referred to page 9 of the specification, lines 6-13, which refer to the art-known existence of a multiplicity of GFP variants. In addition to the documents referred to, applicants point out that a large number of GFP variants in a variety of colors is available commercially and can be purchased from commonly accessed scientific supply houses.

The third factor (labeled IV) is the nature of the invention/state-of-the-art and relative skills of those in the art/predictability or unpredictability of the art.

Again, the basis here appears to be the absence of specifying a ligation step in the claims; this objection has been obviated by amendment.

In view of the foregoing, it is believed that the rejections under 35 U.S.C. § 112, paragraph 1, may be withdrawn.

The Rejection Under 35 U.S.C. § 112, Paragraph 2

This basis for rejection, for the most part appears identical to that set forth under the preceding section. Applicants' response is much the same; this rejection is obviated by amendment to the claims.

The rejection specifically applied to claim 10 is moot as well.

The Rejections Under 35 U.S.C. § 103

Claims 1-3 and 10 and 13 were rejected as assertedly obvious over Chien, *et al.,* in view of Waldo, *et al.* From the text of the objection, it appears that the inclusion of Waldo is related to claims 4 and 11, which are not included in the original statement of rejection. Waldo is stated to "teach GFP fusion proteins for the formation of folding robustness to improve soluble expression in *E. coli*." Claims 4 and 11 thus appear therefore included in the rejection; claim 7 appears to be included as well (see page 10, end of the bridging paragraph).

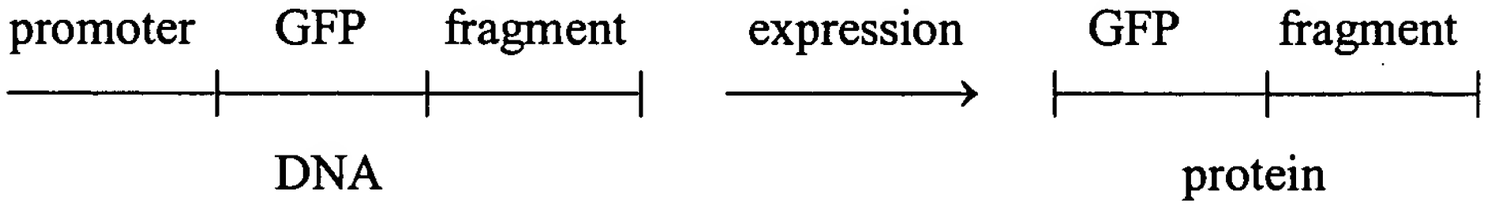
The basis for rejection can be addressed by contrasting the method of the invention with that of the primary document cited, Chien.

Respectfully, it is difficult for applicants to understand the relevance of Chien to the present invention. Chien describes a method to detect the interaction of two proteins intracellularly by attaching to each a portion of a transcriptional activator protein. Neither portion of the transcriptional activator protein can induce transcription by itself; only when the

two proteins containing the two "halves" interact, does the transcriptional activating factor itself come together and become functional. Now that it is functional, it can interact with a reporter system to effect the expression of a reporter gene, such as *lacZ*.

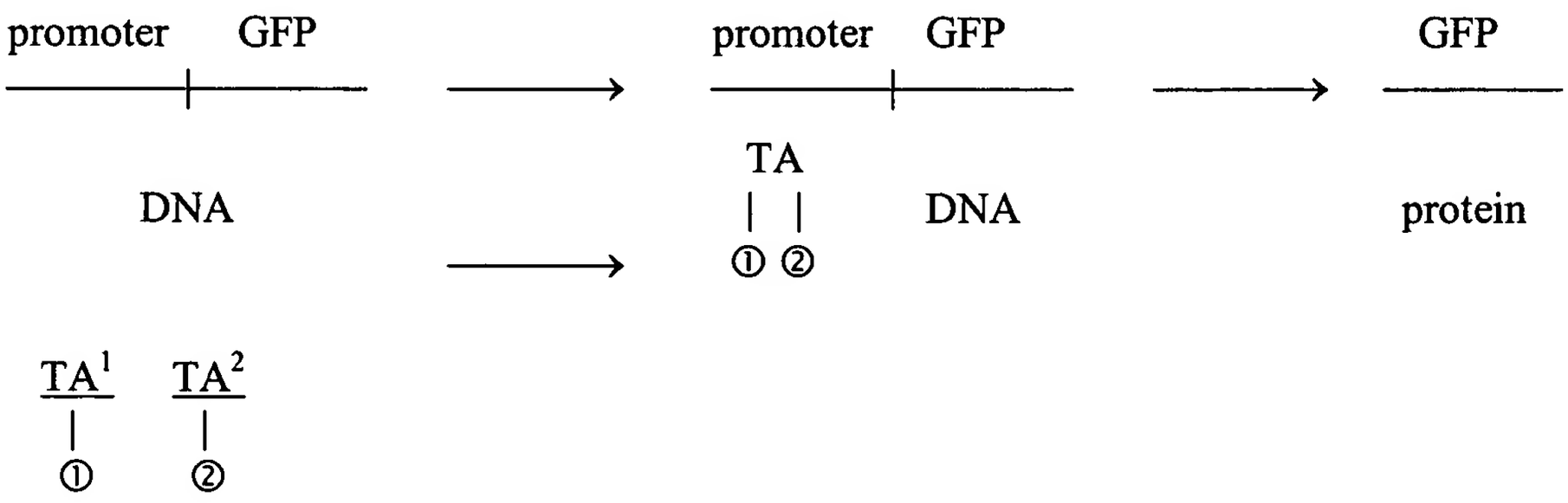
This has nothing to do with the method of the present invention which fuses a functional "reporter" protein to a fragment of a starting protein to be assessed for solubility. Perhaps the following "cartoons" will be helpful, both using, arbitrarily, GFP as the reporter.

Invention Method:



If the protein produced fluoresces, the attached fragment is considered to be a soluble domain; if it does not fluoresce, it is not. The solubility of the domain is roughly proportional to the fluorescence emitted.

Chien:



where

TA^1 + TA^2 = portions of transcription activator
① + ② = interacting proteins

In a somewhat oversimplified sequence, the two portions of the transcription activator (TA) interact with each other when the proteins to which they are coupled (① and ②)

interact, permitting the transcription activator to interact with the promoter resulting in the production of GFP protein.

There appears to be no similarity whatsoever in the two methods, either as to their purpose or as to the steps involved. The invention method is designed to *evaluate the solubility* of a protein or fragment by evaluating the ability of a fusion of the protein or fragment with (in this illustration) GFP to retain its fluorescence; the Chien method is directed to *detecting the interaction of two proteins* by virtue of their ability to reconstitute a transcription activator, thus permitting the production of GFP which, as an isolated protein, will *always* be fluorescent. Applicants see no connection at all between these two methods other than the use of GFP as a reporter protein.

Accordingly, this basis for rejection may properly be withdrawn.

Claims 10-14 were rejected as obvious over Waldo alone.

Waldo is cited in the specification and is at least relevant to the present invention. Waldo prepared fusions of green fluorescent protein with a number of other proteins produced in *E. coli* and observed that the fluorescence emitted by green fluorescent protein is correlated with the appropriate folding of the fused amino acid portion even when detached from GFP. Waldo does not suggest applying this approach to identifying soluble domains that are fragments of a starting protein. There is no suggestion in Waldo to obtain fragments of a protein and test them for solubility by fusing the fragments to GFP and detecting fluorescence either *in vivo* or in a cell-free system. Instead, the DNA fused to DNA encoding GFP by Waldo encodes whole proteins or subunits, which are not further treated to form fragments. For example, as shown in figure 1, Waldo expressed 20 different proteins that are fusions with GFP in *E. coli* which include whole enzymes or subunits such as nucleoside diphosphate kinase, tyrosine tRNA kinase, sulfate

reductase dissimilatory subunit, and the like. Figures 2 and 3 show only whole or subunit proteins as well. This is unlike the present invention which provides for deletion of a cloned DNA fragment encoding a protein and expressing the fragment of the protein. No document has been cited by the Office that makes this suggestion. Accordingly, the rejection over Waldo may also be withdrawn.

CONCLUSION

The rejections under 35 U.S.C. § 112, paragraph 1, appear misplaced as a criticism of claim language rather than rejection for lack of written description or enablement. In any event, the claims have been amended to obviate both this basis for rejection and the rejection made under paragraph 2 of this statutory section. The rejection over Chien appears misplaced, as Chien describes an unrelated method for an unrelated purpose. The rejection over Waldo is in error due to the failure of Waldo to suggest applying fusion techniques to detect soluble domains of, proteins, rather Waldo suggests only evaluation of folding of complete proteins. Accordingly, it is believed that claims 1, 3-7, 10-14 and new claims 15-23 are allowable over the art and passage of these claims to issue is respectfully requested.

If it is believed that a telephone interview would be helpful in resolving issues of claim wording and the like, a telephone call to the undersigned is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No 251002009400.

Respectfully submitted,

Dated: June 15, 2004

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